ATTORNEY DOCKET NO. 05118.0007U4

DECLARATION FOR PATENT APPLICATION

() Original () Supplemental (X) Substitute () PCT

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **"TRANSGENIC ANIMALS THAT PRODUCE HUMAN HEMOGLOBIN"**, which is described and claimed in the specification

(check one) [] which is attached hereto, or
[X] which was filed on October 30, 1997, as United States Application No. 08/961,443 and with amendments through. (if applicable), or
[] in International Application No. PCT/, filed , and as amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) or §365(b) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATIONS: (ENTER BELOW IF APPLICABLE)			PRIORITY CLAIMED (MARK APPROPRIATE BOX BELOW)	
APP. NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	YES	NO

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

ATTORNEY DOCKET NO. 05118.0007U4

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known by me to be material to the patentability of the claims of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS (MARK APPROPRIATE COLUMN BELOW)		
		PATENTED	PENDING	ABANDONED
08/934,385	September 19, 1997		X	
08/888,433	July 7, 1997			X
08/611,542	March 6, 1996			X

Address all telephone calls to David G. Perryman at telephone no. (404) 688-0770.

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NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

ATTORNEY DOCKET NO. 05118.0007U4

Full name of first inventor: Tim M. Townes

Inventor's signature: Tim M. Townes Date: 04/10/02

Residence: 4687 Bridgewater Road, Birmingham, AL 35243

Post Office Address: 4687 Bridgewater Road, Birmingham, AL 35243

Citizenship: U.S.A.

Full name of second inventor: Thomas Ryan

Inventor's signature: T L Ryan Date: 4/10/02

Residence: 1610 Cahaba Rd. Birmingham, Alabama 35223

Post Office Address: 1610 Cahaba Rd. Birmingham, Alabama 35223

Citizenship: U.S.A.

Full name of third inventor: Dominic Ciavatta

Inventor's signature: _____ Date: _____

Residence: 206 Culbreth Park Dr. Chapel Hill , N.C. 27516

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Citizenship: U.S.A.

ATTORNEY DOCKET NO. 05118.0007U4

Full name of first inventor: Tim M. Townes

Inventor's signature: _____ Date: _____

Residence: 4687 Bridgewater Road, Birmingham, AL 35243

Post Office Address: 4687 Bridgewater Road, Birmingham, AL 35243

Citizenship: U.S.A.

Full name of second inventor: Thomas Ryan

Inventor's signature: _____ Date: _____

Residence: 1610 Cahaba Rd. Birmingham, Alabama 35223

Post Office Address: 1610 Cahaba Rd. Birmingham, Alabama 35223

Citizenship: U.S.A.

Full name of third inventor: Dominic Ciavatta

Inventor's signature: Dominic Ciavatta Date: 4-10-02

Residence: 206 Culbreth Park Dr. Chapel Hill , N.C. 27516

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Citizenship: U.S.A.

ATTORNEY DOCKET NO. 05118.0007U4
Serial No. 08/961,443

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Townes, Tim M. et al.

Serial No. 08/961,443

Filed: October 10, 1997

For: TRANSGENIC ANIMALS THAT PRODUCE HEMOGLOBIN

Group Art Unit: 1632

Examiner: Jill Martin

DECLARATION UNDER 37 C.F.R. 1.132

THE TOWNES DECLARATION 2

BOX FEE AMENDMENT
Commissioner for Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

April 10, 2002

Sir:

1. I, Tim Townes, a citizen of the United States, residing at 4687 Bridgewater Rd, Birmingham, Al 35243, declare that:

2. I have a Ph.D. degree in Microbiology from the University of Tennessee in Knoxville. I have been conducting research in the field of Molecular Genetics since 1982 and am a co-author of at least 50 publications relating to hemoglobin and hemoglobin transgenesis. I am currently Professor and Chairman of the Department of Biochemistry and Molecular Genetics at the University of Alabama at Birmingham.

3. I am a co-inventor of the subject matter in United States Patent Application No. 08/961,443.

4. I have reviewed the Office Action dated December 10, 2001, as well as the following publications:

a. Mullins and Mullins, "Perspective Series: Molecular Medicine in Genetically Engineered Animals Transgenesis in the Rat and Larger Mammals," J. Clin. Invest., 98(11) S37-S40 (1996) ("Mullins")

b. Wall et al., "Transgenic Livestock: Progress and Prospects for the Future," Theriogenology 45:57-68 (1996) ("Wall")

c. Kappel et al., "Regulating Gene Expression in Transgenic Animals," Current Opinions In Biotechnology, 3:548-553 (1992) ("Kappel")

d. Strojek and Wagner, "The Use of Transgenic Animal Techniques for Livestock Improvement," Genetic Engineering: Principles and Methods, 10:221-246 Plenum Press, (1988) ("Strojek")

e. Grovsfeld F. et al., "Position-independent, high level expression of the human beta-globin gene in transgenic mice," Cell 51:975-985 (1987) ("Grosvfeld")

f. Paszty et al., "Lethal a-thalassaemia created by gene targeting in mice and its genetic rescue," Nat Genet., 11(1):33-9 (1995) ("Paszty")

g. Ciavatta et al. "Mouse model of human β° thalassemia:Targeted deletion of the mouse β^{maj} - and β^{min}-globin genes in embryonic stem cells," Proc. Natl. Aced. Sci. USA 92:9259-9263 (1995) ("Ciavatta")

h. Rubin et al., Hypoxia-induced in Vivo Sickling of Transgenic Mouse Red Cells," J. Clin. Invest., 87:639-47 (1991) ("Rubin")

i. Fabry et al. "A Second Generation Transgenic Mouse Model Expressing Both Hemoglobin S (HbS) and HbS-Antilles Results in Increased Phenotypic Severity," Blood, 86:2419-28 (1995)

5. After reviewing the Office Action dated December 10, 2001, I understand that the examiner believes that 1) hemoglobin transgene expression is not predictable in mammals other than mice, and 2) switching constructs are required for all hemoglobin transgenes.

6. The Beta Globin Locus Control Region (LCR) sequences provide high level, position independent expression. As referenced in Mullins and Mullins above, LCRs have been used to express transgenes at high levels in species other than mice. In particular, the Beta Globin LCR has been used to produce high levels of human alpha and beta globin in transgenic pigs (See Swanson et al., and Sharma et al., Appendix A and B)).

7. Hemoglobin is an intracellular protein which functions in oxygen delivery. Levels of hemoglobin that vary from 10 to 14 grams per deciliter are consistent with relatively normal physiology and levels as low as 7 grams per deciliter can sustain life. Therefore, relatively wide variations of hemoglobin concentration can be tolerated in mammals. On the other hand, wide variations of the concentrations of secreted proteins are not well tolerated. This is particularly true of hormones. The levels of hormones are tightly regulated in mammals because of their potency. Since small quantities of hormones can have major phenotypic effects because of their very nature, the production of animals expressing transgenic hormones is much less predictable than the production of animals expressing non-hormonal transgenic intracellular proteins. The initial publications of large animal transgenics involved the expression of hormones, and these animals were abnormal. However, large animals that express human hemoglobin are normal. For example, Swanson et al. and Sharma et al, (Appendix A) indicate that transgenic hemoglobin was expressed at physiologically acceptable levels.

8. The production of mice that survive on human hemoglobin is predictive of the survival of larger mammals. The physiology of the mouse is more different from humans than is the physiology of the cow or sheep and humans. The high metabolic rate of the mouse requires efficient oxygen delivery and the oxygen affinity of mouse and humans are significantly different. Therefore, we could not predict that the mouse would survive on human hemoglobin.

However, the fact that mice did survive exclusively on human hemoglobin makes it more predictable that large animals would also survive solely on human hemoglobin.

9. Switching constructs are not required for all hemoglobin transgenes. We used a construct that switches from human gamma (fetal) to human beta (adult) globin during development when producing our mouse model of sickle cell disease. In this case, the production of human fetal hemoglobin is important to inhibit red cell sickling during fetal development and in new born animals. When a normal adult beta globin gene is linked to the LCR, normal adult hemoglobin is synthesized in the fetus and newborn and these animals are normal.

10. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

Tim Townes

04/10/02

Tim Townes, Ph.D. **DATE**

Appendix A.

1. Biotechnology (N Y) 1992 May;10(5):557-9

Production of functional human hemoglobin in transgenic swine.
Swanson ME, Martin MJ, O'Donnell JK, Hoover K, Lago W, Huntress V, Parsons CT, Pinkert CA, Pilder S, Logan JS.
DNX, Inc., Princeton, NJ 08540.

A construct containing the locus control region (LCR) from the human beta globin locus together with two copies of the human alpha 1 gene and a single copy of the human beta A gene was used to obtain three transgenic pigs. The transgenic pigs are healthy, not anemic, and grow at a rate comparable to non-transgenic littermates. All animals expressed the human genes. However, alpha globin was consistently expressed at higher levels than beta globin. Isolation of the human hemoglobin from both porcine hemoglobin and other non-hemoglobin proteins was accomplished by ion exchange chromatography. The purified porcine derived human hemoglobin exhibited an oxygen affinity similar to that of human derived human hemoglobin.

2. Biotechnology (N Y) 1994 Jan;12(1):55-9

An isologous porcine promoter permits high level expression of human hemoglobin in transgenic swine.
Sharma A, Martin MJ, Okabe JF, Truglio RA, Dhanjal NK, Logan JS, Kumar R.
DNX Biotherapeutics Inc, Princeton, NJ 08540.
We describe isologous promoter replacement as an approach to permit high level expression of human hemoglobin in transgenic swine. We linked the human beta globin genomic coding region to the porcine beta globin promoter and used this fusion gene in an expression construct containing the human beta locus control region and the human alpha and epsilon genes to produce transgenic pigs. The highest level of expression was 24% human (32g/liter) and 30% human alpha/pig beta hybrid (40g/liter) hemoglobin in one transgenic pig. This pig was bred to a non-transgenic animal resulting in the transmission of high level human hemoglobin expression to 5 of 12 progeny.